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(54) Title: IL-13 CONJUGATED IMMUNOTOXIN OR CYTOTOXIN AND USES THEREOF

(57) Abstract: The invention provides a method for inducing an immune response against a disease in a human, which method comprises providing a conjugate comprising a portion of interleukin 13 (IK-13) fused to an immunogen, and administering the conjugate to the human, whereupon an immune response to the disease is induced. The invention also provides a method for treating a disease in a human, which method comprises preparing an antibody raised against a conjugate comprising a portion of IL-13 fused to an immunogen, and administering the antibody to the human, whereupon the disease is treated.

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IL-13 CONJUGATED IMMUNOTOXIN OR CYTOTOXIN AND USES THEREOF

FIELD OF THE INVENTION

[0001] This invention pertains to a conjugate comprising a portion of interleukin 13 (IL-13) fused to an immunogen, and methods of using same.

BACKGROUND OF THE INVENTION

[0002] The overexpression of immunomodulator molecules, such as cytokines and cytokine receptors, has been implicated in the development of a growing number of human diseases, including some forms of cancer, allergies, and certain infectious diseases.

[0003] In particular, overexpression of the cytokine interleukin 13 (IL-13) has been shown to play a central role in the pathogenesis of allergic airway disease, such as asthma, by inducing airway hypersensitivity and infiltration by inflammatory cells (see, e.g., Blease et al., *J. Immunol.*, *166*, 5219-5224 (2001), Blease et al., *J. Immunol.*, *167*, 6583-6592 (2001), and Walter et al., *J. Immunol.*, *167*, 4668-4675 (2001)). According to the National Institute of Allergies and Infectious Disease (NIAID) and the American Lung Association, there are 17 million asthma sufferers in the United States. It is considered a serious illness, which is increasing in prevalence, characterized by one or more symptoms including episodic shortness of breath, wheezing, coughing, and chest tightness.

[0004] Asthma is currently treated with non-specific anti-inflammatory drugs, beta agonists or other bronchodilators or steroids that control airway inflammation, such as corticosteroids. Corticosteroids, however, do not specifically target the lung, and therefore often produce systemic adverse effects (see, e.g., International Patent Application WO 01/62287).

[0005] In addition to disease associated with overexpression of IL-13, IL-13 receptor (IL-13R) expression has been associated with the development and/or progression of certain cancers. Specifically, overexpression of IL-13R has been observed in tumor cell lines derived from glioblastoma (e.g., malignant glioblastoma multiforme (GBM)), anaplastic astrocytoma (AA), Kaposi sarcoma (KS) (such as AIDS-associated KS), renal cell carcinoma (RCC), prostate cancer, head and neck cancer (SCCHN), and ovarian cancer, which have been found to express high- to

intermediate-affinity IL-13R on the cell surface. Also, some breast cancer cells can be stimulated to divide and grow when a protein produced naturally in the body binds to HER2, a receptor on the cell surface. Thus, it is believed that overexpression of IL-13R promotes tumor growth via increased signaling through IL-13R. The treatment of IL-13R-expressing cancers, however, has been limited by the inability to selectively target treatment to the tumor site without adversely affecting non-tumor tissue.

[0006] Thus, in view of the above, there remains a need for methods to effectively treat diseases associated with IL-13 expression, IL-13R expression, or cancers, asthma, and infections.

BRIEF SUMMARY OF THE INVENTION

[0007] The invention provides a method for inducing an immune response against a disease in a human, which method comprises providing a conjugate comprising a portion of interleukin 13 (IL-13) fused to an immunogen, and administering the conjugate to the human, whereupon an immune response to the disease is induced. The invention also provides a method for treating a disease in a human, which method comprises preparing an antibody raised against a conjugate comprising a portion of IL-13 fused to an immunogen, the cytotoxicity of which has been destroyed, and administering the antibody to the human, whereupon the disease is treated. These and other advantages of the invention, as well as additional inventive features, will be apparent from the description of the invention provided herein.

BRIEF DESCRIPTION OF THE DRAWINGS

[0008] Figure 1 depicts the production of antibodies directed against a conjugate comprising a portion of IL-13 fused to the *P. aeruginosa* exotoxin A.

DETAILED DESCRIPTION OF THE INVENTION

[0009] In one aspect, the invention provides a method for inducing an immune response against a disease in a human. The method comprises (a) providing a conjugate comprising a portion of interleukin 13 (IL-13) fused to an immunogen and

(b) administering the conjugate to the human, whereupon an immune response to the disease is induced.

[0010] The inventive method involves administration of a conjugate to a human. The term "conjugate," as used herein, refers to a molecule comprising an immunogen molecule (e.g., a tumor antigen, a bacteria or plant antigen or toxin, a radionuclide, or a chemotherapeutic drug), chemically linked to a molecule that selectively targets the immunogen to a specific cell. The targeting molecule of the conjugate typically is comprised of a molecule that binds to a specific receptor or antigen that is expressed on the surface of a particular cell type, such as a tumor-specific antigen. In the context of the inventive method, the conjugate comprises a portion of IL-13 fused to the immunogen. Any portion of IL-13 can be employed in the conjugate, so long as the portion of IL-13 retains the ability to bind to a corresponding IL-13 receptor. In this respect, the portion of IL-13 employed in the conjugate preferably exhibits an affinity constant for an IL-13 receptor that is at least 1/10,000 of the affinity of native IL-13. The portion of IL-13 can be isolated from natural sources, synthetically produced, or semi-synthetically produced using methods known in the art. In this respect, the portion of IL-13 can include, for example, IL-13 itself or derivatives thereof. Suitable IL-13 derivatives include genetically constructed derivatives and chemical derivatives. Genetic derivatives of IL-13 are generated by introducing, for example, truncations, deletions, insertions, or other mutations into the nucleic acid sequence encoding IL-13, so long as the polypeptide produced therefrom retains a suitable binding affinity for an IL-13 receptor. Similarly, chemical modifications of IL-13 include any chemical modifications that do not preclude binding of the targeting moiety to an IL-13 receptor in the conjugate. Indeed, IL-13 can be modified in any suitable manner, so long as the portion of IL-13 employed in the conjugate molecule can bind to an IL-13 receptor with a desired affinity.

[0011] The portion of IL-13 employed in the conjugate molecule can bind any suitable receptor for IL-13. Suitable receptors for IL-13 include the α-chain of the interleukin 4 (IL-4) receptor, the α1 IL-13 receptor (IL-13Rα1), and the α2 IL-13 receptor (IL-13Rα2). The IL-13 receptor, however, is not limited to these exemplary receptors. Indeed, any receptor that binds to IL-13 with sufficient affinity is within the scope of the inventive method.

[0012] In accordance with the inventive method, the portion of IL-13 is fused to the immunogen. The term "immunogen," as used herein, refers to any substance (typically and preferable a protein) which is capable of inducing an immune response in an animal, preferably a human. Any suitable immunogen can be employed, such as bacterial or viral antigens, bacterial or viral toxins, tumor antigens, and the like. Such an immunogen can be fused to the portion of IL-13, and, as such, the invention includes the use of IL-13 as a carrier for a vaccine (i.e., the immunogen).

[0013] A preferred immunogen for use in the conjugate includes a toxin, such as a bacterial toxin, a viral toxin, a fungal toxin, or a toxin produced by a parasitic agent. Preferably, the toxicity of such a toxin is reduced or eliminated, for use in the context of the present invention. Suitable toxins for use as conjugates include, for example, toxins produced by pathogenic organisms (e.g., bacteria, viruses, fungi, and parasites) and plants, cytotoxic drugs (e.g., chemotherapeutic drugs), and radionuclides. Preferably, the toxin is a bacterial toxin. Suitable bacterial toxins include, for example, *Aeromonas hydrophila* aerolysin toxin, *Escherichia coli* hemolysin toxin, the enterotoxins, exfoliative toxins, toxic-shock toxins, and α-toxin of *Staphyloccocus aureus*, *Streptococcus pyogenes* streptolysin O toxin and pyrogenic exotoxins, diphtheria toxin, *Bacillus anthracis* edema factor and lethal factor, *Bordetella pertussis* dermonecrotic toxin and pertussis toxin, cholera toxin, tetanus toxin, and *Psuedomonas aeruginosa* exotoxin A. Preferably, the toxin is a *Psuedomonas* exotoxin, most preferably *P. aeruginosa* exotoxin A. Most preferably, the conjugate is designated IL13-PE38QQR (see, e.g., WO 03/039600, Puri et al., *Blood, 87(10)*, 4333-9 (1996), Husain et al., *Blood, 95(11)*, 3506-13 (2000), and Husain et al., *Int. J. Cancer, 92*, 168-75 (2001)). Additional toxins that can be used in the conjugate are listed in Table 1 (Schmitt, et al., *Emerging Infectious Diseases, 5(2)*, 1-14 (1999)), along with disease states that can be treated in accordance with the inventive method wherein the conjugate includes the respective toxin or portion or derivative thereof.

Table 1.

Organism/toxin	Mode of action	Target	Disease	Toxin Implicated in Disease[b]
Toxins that Damage Membranes				
Aeromonas hydrophila/aerolysin	Pore-former	Glycophorin	Diarrhea	(yes)
Clostridium perfringens/ perfringolysin O	Pore-former	Cholesterol	Gas gangrene[c]	?
Escherichia coli/ hemolysin[d]	Pore-former	Plasma membrane	UTIs	(yes)
Listeria monocytogenes/ listeriolysin O	Pore-former	Cholesterol	Foodborne systemic illness meningitis	(yes)
Staphylococcus aureus/α-toxin	Pore-former	Plasma membrane	Abcesses[c]	(yes)
Streptococcus pneumoniae/ pneumolysin	Pore-former	Cholesterol	Pneumonia[c]	(yes)
Streptococcus pyogenes/ streptolysin O	Pore-former	Cholesterol	Strep throat Sf[c]	?
Toxins that Inhibit Protein Synthesis				
Corynebacterium diphtheriae/ diphtheria toxin	ADP-ribosyltransferase	Elongation factor 2	Diphtheria	yes
E. coli/*Shigella dysenteriae*/ Shiga toxins	N-glycosidase	28S rRNA	HC and HUS	yes
Pseudomonas aeruginosa/ exotoxin A	ADP-ribosyltransferase	Elongation factor 2	Pneumonia[c]	(yes)
Toxins that Activate Second Messenger Pathways				

Organism/toxin	Mode of action	Target	Disease	Toxin Implicated in Disease[b]
E.coli				
CNF	Deamidase ADP-	Rho G-proteins	UTIs	?
LT	ribosyltransferase	G-proteins	Diarrhea	yes
ST[d]	Stimulates guanylate cyclase	guanylate cyclase receptor	Diarrhea	yes
CLDT[d]	G2 block	Unknown	Diarrhea	(yes)
EAST	ST-like?	Unknown	Diarrhea	?
Bacillus anthracis/ edema factor	Adenylate cyclase	ATP	Anthrax	yes
Bordetella pertussis				
dermonecrotic toxin	Deamidase	Rho G-proteins	Rhinitis	(yes)
pertussis toxin	ADP-ribosyltransferase	G-protein(s)	Pertussis	yes
Clostridium botulinum				
C2 toxin	ADP-ribosyltransferase	Monomeric G-actin	Botulism	?
C3 toxin	ADP-ribosyltransferase	Rho G-protein	Botulism	?
Clostridium difficile				
toxin A	Glucosyltransferase	Rho G-protein(s)	Diarrhea/PC	(yes)
toxin B	Glucosyltransferase	Rho G-protein(s)	Diarrhea/PC	?
Vibrio cholerae/ cholera toxin	ADP-ribosyltransferase	G-protein(s)	Cholera	yes
Toxins that Activate Immune Response				

Organism/toxin	Mode of action	Target	Disease	Toxin Implicated in Disease[b]
Staphylococcus aureus				
Enterotoxins	Superantigen	TCR and MHC II	Food poisoning[c]	yes
exfoliative toxins	Superantigen (and serine protease)	TCR and MHC II	SSS[c]	yes
toxic-shock toxin	Superantigen	TCR and MHC II	TSS[c]	yes
S. pyogenes/pyrogenic exotoxins	Superantigens	TCR and MHC II	SF/TSS[c]	yes
Protease Toxins				
B. anthracis/lethal factor	Metalloprotease	MAPKK1/MAPKK2	Anthrax	yes
C. botulinum/ neurotoxins A-G	Zinc-metalloprotease	VAMP/synaptobrevin SNAP-25 syntaxin	Botulism	yes
Clostridium tetani/ tetanus toxin	Zinc-metalloprotease	VAMP/synaptobrevin	Tetanus	yes

[b] Yes: Strong casual relationship between toxin
(Yes): Role in pathogenesis has been shown in animal model or appropriate cell culture
[c] Other diseases are also associated with the organism
[d] Toxin is also produced by other genera of bacteria
? Unknown

[0014] In a preferred embodiment of the invention, where a toxin is employed as the immunogen, the cytotoxicity of the toxin is reduced or destroyed, such that administration of the conjugate does not result in the destruction of cells which express an IL-13 receptor and thus, bind to the conjugate. The cytotoxicity of the toxin can be destroyed using standard molecular biology and recombinant DNA techniques, such as those described in, for example, Sambrook et al. (eds.), *Molecular Cloning, A Laboratory Manual*, 3rd Edition, Cold Spring Harbor Laboratory Press, New York (2001)). In this respect, for example, the nucleic acid sequence encoding the toxin can be modified or engineered to introduce mutations (e.g., deletions, insertions, inversions) in domains that are required for cytotoxicity.

[0015] The portion of IL-13 and the immunogen can be joined by any suitable means. For example, the portion of IL-13 and the immunogen can be joined chemically such as through cysteine disulfide bonds or other chemical conjugation

methods. Desirably, the portion of IL-13 and the immunogen are joined at the genetic level in a recombinant fusion protein, such as is described in U.S. Patents 5,614,191 and 5,919,456.

[0016] The inventive method desirably induces an immune response against a disease associated with expression of IL-13 or a receptor for IL-13, most preferably overexpression of IL-13 or a receptor for IL-13. In this respect, the invention provides for the use of IL-13 conjugates as a vaccine, which can be employed prophylactically against various mammalian diseases and infections. The inventive method can induce an immune response against any suitable disease associated with expression of IL-13 or a receptor for IL-13. Such diseases include, for example, allergic airway disease (e.g., asthma) and certain types of cancer (e.g., malignant glioblastoma multiforme (GBM), anaplastic astrocytoma (AA), Kaposi sarcoma (KS), renal cell carcinoma (RCC), prostate cancer, head and neck cancer, ovarian cancer, and breast cancer). The inventive method, however, is not limited to these exemplary diseases. Any suitable disease associated with expression of IL-13 or an IL-13 receptor is within the scope of the invention.

[0017] In addition to diseases associated with IL-13 or an IL-13 receptor, the inventive method can be used to induce an immune response against a disease caused by an infectious agent, and therefore serve as a vaccine against such agents. The infectious agent can be any infectious agent that is pathogenic (i.e., disease-causing) in a human. Suitable infectious agents include, for example, a bacterium, a virus, a fungus, or a parasite. In this embodiment, the conjugate comprises an immunogen associated with or produced by the infectious agent (e.g., a toxin, as discussed herein), such that, when administered to a human in accordance with the inventive method, the conjugate elicits an immune response against the immunogen, thereby resulting in neutralization of the infectious agent, treatment of the disease, and protection from subsequent infection.

[0018] In accordance with the inventive method, the immune response induced by administration of the conjugate described herein can be either a humoral immune response or a cell-mediated immune response (see, e.g., C.A. Janeway et al. (eds.), *Immunobiology*, 5th Ed., Garland Publishing, New York, NY (2001)). Preferably, the immune response is a humoral immune response, which is mediated by antibodies

produced by B-lymphocytes, or "B-cells." The term "antibody" (also known in the art as an "immunoglobulin"), as used herein, refers to a molecule having a specific amino acid sequence, by virtue of which it interacts only with the antigen that induced its synthesis in cells of the lymphoid series (especially plasma cells), or with an antigen closely related to it. The term "antigen" refers to any molecule that can bind specifically to an antibody. Antibodies typically are produced in response to infection or immunization, which bind to and neutralize pathogens, or prepare pathogens for uptake and destruction by phagocytes (see, e.g., Janeway et al., *supra*). The general structure and function of antibody molecules are well known in the art.

[0019] Thus, in accordance with the inventive method, the immune response produced by administration of the conjugate to a human comprises the production of one or more antibodies. When the inventive method induces an immune response against a disease associated with IL-13 expression or IL-13R expression as described above, the one or more antibodies preferably are directed against IL-13. The one or more antibodies directed against IL-13 desirably is specific to the portion of IL-13 included in the conjugate molecule, and will bind to IL-13 molecules in the human. Binding of the one or more antibodies to IL-13 inhibits binding of IL-13 to a receptor for IL-13, thereby preventing signaling by IL-13 through IL-13R. When the inventive method induces an immune response against an infectious agent (e.g., a bacterium, virus, fungus, etc.) or a cancerous cell, the conjugate comprises an immunogen produced by the infectious agent or cancerous cells, and the one or more antibodies preferably are directed against the immunogen. In this manner, binding of the one or more antibodies to the immunogen can neutralize the infectious agent or cancerous cell. Antibody production and antibody specificity can be measured using any suitable method known in the art, such as for example, radioimmunoassay (RIA), ELISA, Western blot, immunoprecipitation, and competitive inhibition assays (see, e.g., Janeway et al., *supra,* and U.S. Patent Application Publication No. 2002/0197266 A1).

[0020] In another aspect, the invention also provides a method for treating a disease in a human, which comprises (a) preparing an antibody raised against a conjugate comprising a portion of IL-13 fused to an immunogen, and (b) administering the antibody to the human, whereupon the disease is treated.

Descriptions of the conjugate (i.e., the portion of IL-13 fused to the immunogen), the antibody, the disease, and components thereof, set forth above in connection with other embodiments of the invention also are applicable to those same aspects of the aforesaid method of treating a disease.

[0021] The inventive method for treating a disease in a human involves preparing an antibody raised against the conjugate described herein. Typically and preferably, the antibody is an "isolated antibody" (or fragment thereof). An "isolated" antibody (or fragment thereof) refers to at least one antibody molecule that has been isolated, or is otherwise free of, the bulk of the total antibodies circulating in the bloodstream of an animal (preferably a mammal, and more preferably a human). Total isolation from all other antibodies, however, is not necessary. Indeed, the antibody can be polyclonal in some embodiments. In other words, an antibody is "isolated" if it has been changed or removed from its natural *in vivo* environment. Methods of generating antibodies using purified polypeptides or synthetic oligonucleotides are known in the art. Generally, such methods typically involve administering an oligonucleotide or polypeptide encoding an antigenic determinant mixed with an adjuvant to an organism (e.g., a rabbit, mouse, sheep, etc.), such that antibodies directed against the antigen are produced by the organism (see, e.g., Harlow and Lane (eds.), *Antibodies: A Laboratory Manual*, CSH Press (1988), Salvatore et al., *Biochem. Biophys. Res. Comm.*, *294*, 813-817 (2002), and U.S. Patents 5,776,457 and 5,614,191). Thus, in the context of the inventive method, the polypeptide encoding an antigenic determinant preferably is the conjugate molecule. Specific antibodies raised against the immunizing antigen (i.e., the conjugate) can be isolated and purified from animal serum using any suitable method known in the art. Such methods include, for example, affinity chromatography, in which immunized serum is applied to beads loaded in a column that are covalently bound to the antigen of interest. Non-specific antibodies and other serum proteins are washed away, leaving only antigen-specific antibodies bound to the antigen coated beads, which are eluted by adjusting the pH, temperature, or salt concentration of the reaction conditions. Other suitable methods for antibody isolation and purification are disclosed in, for example, Published U.S. Patent Application No. 20020197266/A1, U.S. Patent 5,776,457, and Janeway et al., *supra*.

[0022] The antibody prepared in accordance with the inventive method can be any immunoglobulin, any immunoglobulin fragment, such as Fab, $F(ab')_2$, dsFv, sFv, multimeric antibodies (e.g., diabodies and triabodies), or immunoglobulin chimera, so long as it is raised against one or both portions of the conjugate. One of ordinary skill in the art will appreciate that the generation and selection of an appropriate antibody will depend upon the type of disease to be treated. In this regard, if the disease to be treated is associated with expression of IL-13R or IL-13 (e.g., gliomas or asthma, respectively), then the antibody preferably is prepared such that it is directed against the IL-13 portion of the conjugate. In this manner, the antibody (or fragment thereof) binds to IL-13 when administered to a human, thereby inhibiting binding of IL-13 to a receptor for IL-13. Alternatively, if the disease to be treated is caused by a cancerous cell or an infectious agent (e.g., a bacterium, virus, fungus, or parasite), then the antibody preferably is prepared such that it is directed against the immunogen portion of the conjugate (where the immunogen is derived from a protein or other material produced by the cancerous cell or infectious agent). In this manner, the antibody (or fragment thereof) binds to the conjugate containing the immunogen when administered to a human, thereby neutralizing the cancerous cell or infectious agent.

[0023] The antibody can be polyclonal or monoclonal, but is most preferably a monoclonal antibody. As used herein, "polyclonal" antibodies refer to heterogeneous populations of antibody, typically contained in the sera of immunized animals. "Monoclonal" antibodies refer to homogenous populations of antibody molecules that are specific to a particular antigenic epitope. Monoclonal antibodies are typically produced by a single clone of B lymphocytes ("B cells"). Monoclonal antibodies may be obtained using a variety of techniques known to those skilled in the art, including standard hybridoma technology (see, e.g., Köhler and Milstein, *Eur. J. Immunol.*, *5*, 511-519 (1976), Harlow and Lane, *supra*, and Janeway et al., *supra*). In brief, the hybridoma method of producing monoclonal antibodies typically involves injecting any suitable animal, typically and preferably a mouse, with an antigen (i.e., an "immunogen"). The animal is subsequently sacrificed, and B cells isolated from its spleen are fused with human myeloma cells. A hybrid cell is produced (i.e., a "hybridoma"), which proliferates indefinitely and continuously secretes high titers of an antibody with the desired specificity *in vitro*. Any appropriate method known in

the art can be used to identify hybridoma cells that produce an antibody with the desired specificity. Such methods include, for example, enzyme-linked immunosorbent assay (ELISA), Western blot analysis, and radioimmunoassay. The population of hybridomas is screened to isolate individual clones, each of which secretes a single antibody species against the antigen. Because each hybridoma is a clone derived from fusion with a single B cell, all the antibody molecules it produces are identical in structure, including their antigen binding site and isotype. Monoclonal antibodies also may be generated using other suitable techniques including EBV-hybridoma technology (see, e.g., Haskard and Archer, *J. Immunol. Methods*, *74*(2), 361-67 (1984), and Roder et al., *Methods Enzymol.*, *121*, 140-67 (1986)), or bacteriophage vector expression systems (see, e.g., Huse et al., *Science*, *246*, 1275-81 (1989)). To prepare monoclonal antibody fragments, recombinant methods typically are employed.

[0024] The monoclonal antibody can be isolated from or produced in any suitable animal, but is preferably produced in a mammal, more preferably a mouse, and most preferably a human. Methods for producing an antibody in mice are well known to those skilled in the art and are described herein. With respect to human antibodies, one of ordinary skill in the art will appreciate that polyclonal antibodies can be isolated from the sera of human subjects vaccinated or immunized with an appropriate antigen. Alternatively, human antibodies can be generated by adapting known techniques for producing human antibodies in non-human animals such as mice (see, e.g., U.S. Patents 5,545,806, 5,569,825, and 5,714,352, and U.S. Patent Application Publication No. 2002/0197266 A1).

[0025] While being the ideal choice for therapeutic applications in humans, human antibodies, particularly human monoclonal antibodies, typically are more difficult to generate than mouse monoclonal antibodies. Mouse monoclonal antibodies, however, induce a rapid host antibody response when administered to humans, which can reduce the therapeutic or diagnostic potential of the mouse antibody. To circumvent these complications, a monoclonal antibody preferably is not recognized as "foreign" by the human immune system. To this end, phage display can be used to generate the antibody. In this regard, phage libraries encoding antigen-binding variable (V) domains of antibodies can be generated using standard molecular

biology and recombinant DNA techniques (see, e.g., Sambrook et al. *supra*). Phage encoding a variable region with the desired specificity are selected for specific binding to the desired antigen, and a complete human antibody is reconstituted comprising the selected variable domain. Nucleic acid sequences encoding the reconstituted antibody are introduced into a suitable cell line, such as a myeloma cell used for hybridoma production, such that human antibodies having the characteristics of monoclonal antibodies are secreted by the cell (see, e.g., Janeway et al., *supra*, Huse et al., *supra*, and U.S. Patent 6,265,150). Alternatively, monoclonal antibodies can be generated from mice that are transgenic for specific human heavy and light chain immunoglobulin genes. Such methods are known in the art and described in, for example U.S. Patents 5,545,806 and 5,569,825, and Janeway et al., *supra*. Most preferably the antibody is a humanized antibody. As used herein, a "humanized" antibody is one in which the complementarity-determining regions (CDR) of a mouse monoclonal antibody, which form the antigen binding loops of the antibody, are grafted onto the framework of a human antibody molecule. Owing to the similarity of the frameworks of mouse and human antibodies, it is generally accepted in the art that this approach produces a monoclonal antibody that is antigenically identical to a human antibody but binds the same antigen as the mouse monoclonal antibody from which the CDR sequences were derived. Methods for generating humanized antibodies are well known in the art and are described in detail in, for example, Janeway et al., *supra*, U.S. Patents 5,225,539, 5,585,089 and 5,693,761, European Patent No. 0239400 B1, and United Kingdom Patent No. 2188638. Humanized antibodies can also be generated using the antibody resurfacing technology described in U.S. Patent 5,639,641 and Pedersen et al., *J. Mol. Biol.*, *235*, 959-973 (1994). While the antibody employed in the inventive method most preferably is a humanized monoclonal antibody, a human monoclonal antibody or a mouse monoclonal antibody, as described above, are also within the scope of the invention.

[0026] Antibody fragments that have at least one antigen binding site, and thus recognize and bind to IL-13 or the immunogen (preferably a toxin, as herein described), also are within the scope of the invention. In this respect, proteolytic cleavage of an intact antibody molecule can produce a variety of antibody fragments that retain the ability to recognize and bind antigens. For example, limited digestion

of an antibody molecule with the protease papain typically produces three fragments, two of which are identical and are referred to as the Fab fragments, as they retain the antigen binding activity of the parent antibody molecule. Cleavage of an antibody molecule with the enzyme pepsin normally produces two antibody fragments, one of which retains both antigen-binding arms of the antibody molecule, and is thus referred to as the $F(ab')_2$ fragment. A single-chain variable region fragment (sFv) antibody fragment, which consists of a truncated Fab fragment comprising the variable (V) domain of an antibody heavy chain linked to a V domain of a light antibody chain via a synthetic peptide, can be generated using routine recombinant DNA technology techniques (see, e.g., Janeway et al., *supra*). Similarly, disulfide-stabilized variable region fragments (dsFv) can be prepared by recombinant DNA technology (see, e.g., Reiter et al., *Protein Engineering, 7*, 697-704 (1994)). Antibody fragments of the present invention, however, are not limited to these exemplary types of antibody fragments. Any suitable antibody fragment that recognizes and binds to IL-13 and/or a toxin produced by an infectious agent can be employed. Antibody-antigen binding can be assayed using any suitable method known in the art, such as, for example, radioimmunoassay (RIA), ELISA, Western blot, immunoprecipitation, and competitive inhibition assays (see, e.g., Janeway et al., *supra,* and U.S. Patent Application Publication No. 2002/0197266 A1).

[0027] In addition, the antibody can be a chimeric antibody. By "chimeric" is meant that the antibody comprises at least two immunoglobulins, or fragments thereof, obtained or derived from at least two different species (e.g., two different immunoglobulins, a human immunoglobulin constant region combined with a murine immunoglobulin variable region). In the context of the inventive method, a particularly preferred chimeric antibody comprises an antibody (or fragment thereof) directed against IL-13 fused or otherwise linked to an antibody (or fragment thereof) directed against an immunogen (e.g., a toxin) produced by an infectious agent, such as those describe herein.

[0028] The antibody (or antibody fragment) may be of any immunoglobulin isotype. The term "isotype," as is used in the art, typically describes the class, subclass, light chain type and subtype of an antibody. One of ordinary skill in the art will appreciate that the five major human immunoglobulin isotypes are

immunoglobulin M (i.e., IgM), IgD, IgG, IgA, and IgE, which are typically defined by the structure of the constant regions of the antibody heavy chain. The light chain of a human antibody molecule is typically classified in the art as either a lambda (λ) chain or a kappa (κ) chain. IgG antibodies can be subdivided further into four subtypes (i.e., IgG1, IgG2, IgG3, and IgG4), whereas IgA antibodies typically are subdivided into two subtypes (i.e., IgA1 and IgA2).

[0029] Using any of the methods described herein, one of ordinary skill in the art will appreciate that an animal can be immunized to produce an antibody specific for IL-13 and/or the immunogen by administering to the animal a conjugate comprising a portion of interleukin 13 (IL-13) fused to an immunogen. Alternatively, a gene transfer vector comprising a nucleic acid sequence encoding the conjugate can be generated and administered to an animal using any suitable method known in the art, such that the portion of IL-13 and the immunogen are produced within the animal, resulting in an antibody response against IL-13 and/or the toxin within the animal.

[0030] The invention involves administering to a human the conjugate comprising a portion of interleukin 13 (IL-13) fused to an immunogen, as herein described, or the antibody raised against the conjugate. In such applications, the inventive method can be used alone or adjunctively as part of a treatment for any of a number of diseases, such as asthma, cancer, or infectious diseases as discussed above. For use *in vivo*, the conjugate or antibody raised against the conjugate desirably is formulated into a composition comprising a physiologically acceptable carrier. Any suitable physiologically acceptable carrier can be used within the context of the invention, and such carriers are well known in the art.

[0031] The carrier typically will be liquid, but also can be solid, or a combination of liquid and solid components. The carrier desirably is physiologically acceptable (e.g., a pharmaceutically or pharmacologically acceptable) carrier (e.g., excipient or diluent). Physiologically acceptable carriers are well known and are readily available. The choice of carrier will be determined, at least in part, by the location of the target tissue and/or cells, and the particular method used to administer the composition. In terms of using polypeptide therapeutics as active ingredients, the technology of U.S. Patents 4,608,251, 4,601,903, 4,559,231, 4,559,230, and 4,596,792, each incorporated herein by reference, can be used.

[0032] Typically, such compositions can be prepared as injectables, either as liquid solutions or suspensions; solid forms suitable for using to prepare solutions or suspensions upon the addition of a liquid prior to injection can also be prepared; and the preparations can also be emulsified. The pharmaceutical forms suitable for injectable use include sterile aqueous solutions or dispersions; formulations including sesame oil, peanut oil or aqueous propylene glycol; and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersions. In all cases the form must be sterile and must be fluid to the extent that easy syringability exists. It must be stable under the conditions of manufacture and storage and must be preserved against the contaminating action of microorganisms, such as bacteria and fungi. Solutions of the active compounds as free base or pharmacologically acceptable salts can be prepared in water suitably mixed with a surfactant, such as hydroxycellulose. Dispersions can also be prepared in glycerol, liquid polyethylene glycols, and mixtures thereof and in oils. Under ordinary conditions of storage and use, these preparations contain a preservative to prevent the growth of microorganisms.

[0033] The conjugate or the antibody raised against the conjugate for use in the present invention can be formulated into a composition in a neutral or salt form. Pharmaceutically acceptable salts include the acid addition salts (formed with the free amino groups of the protein) and which are formed with inorganic acids such as, for example, hydrochloric or phosphoric acids, or such as organic acids as acetic, oxalic, tartaric, mandelic, and the like. Salts formed with the free carboxyl groups also can be derived from inorganic bases such as, for example, sodium, potassium, ammonium, calcium, or ferric hydroxides, and such organic bases as isopropylamine, trimethylamine, histidine, procaine and the like.

[0034] The composition can further comprise any other suitable components, especially for enhancing the stability of the composition and/or its end-use. Accordingly, there is a wide variety of suitable formulations of the composition of the invention. The following formulations and methods are merely exemplary and are in no way limiting.

[0035] Formulations suitable for administration via inhalation include aerosol formulations. The aerosol formulations can be placed into pressurized acceptable

propellants, such as dichlorodifluoromethane, propane, nitrogen, and the like. They also can be formulated as non-pressurized preparations, for delivery from a nebulizer or an atomizer.

[0036] Formulations suitable for parenteral administration include aqueous and non-aqueous, isotonic sterile injection solutions, which can contain anti-oxidants, buffers, bacteriostats, and solutes that render the formulation isotonic with the blood of the intended recipient, and aqueous and non-aqueous sterile suspensions that can include suspending agents, solubilizers, thickening agents, stabilizers, and preservatives. The formulations can be presented in unit-dose or multi-dose sealed containers, such as ampules or vials, and can be stored in a freeze-dried (lyophilized) condition requiring only the addition of a sterile liquid excipient, for example, water, for injections immediately prior to use. Extemporaneous injection solutions and suspensions can be prepared from sterile powders, granules, and tablets of the kind previously described. In a preferred embodiment of the invention, the conjugate or antibody (or antibody fragment) raised against the conjugate is formulated for injection. In this regard, the formulation desirably is suitable for intratumoral administration, intravenous injection, intraperitoneal injection, subcutaneous injection, and the like.

[0037] Formulations suitable for anal administration can be prepared as suppositories by mixing the active ingredient with a variety of bases such as emulsifying bases or water-soluble bases. Formulations suitable for vaginal administration can be presented as pessaries, tampons, creams, gels, pastes, foams, or spray formulas containing, in addition to the active ingredient, such carriers as are known in the art to be appropriate.

[0038] In addition, the composition can comprise additional therapeutic or biologically-active agents. For example, therapeutic factors useful in the treatment of a particular indication can be present. Factors that control inflammation, such as ibuprofen or steroids, can be part of the composition to reduce swelling and inflammation associated with *in vivo* administration of the of the conjugate or the antibody (or antibody fragment) raised against the conjugate and physiological distress. Immune system suppressors can be administered with the composition to reduce any immune response to the conjugate or antibody itself or associated with a

disorder. Alternatively, immune enhancers can be included in the composition to upregulate the body's natural defenses against disease. Moreover, cytokines can be administered with the composition to attract immune effector cells to a disease (e.g., tumor) site.

EXAMPLE

[0039] This example further illustrates the invention but, of course, should not be construed as in any way limiting its scope. This example demonstrates a method of inducing an immune response against a conjugate comprising a portion of IL-13 fused to an immunogen.

[0040] The immunogenicity of IL13-PE38QQR, using convection enhanced delivery (CED), was evaluated. An ELISA assay, using a validated qualitative sandwich enzyme immunoassay technique, measured the total amount of IgG antibodies to IL13-PE38QQR. Firstly, patient serum samples were collected before and after intracerebral infusion of IL13-PE38QQR. Secondly, the serum samples were diluted (1:200) and were then incubated with hIL13-PE38QQR, which was immobilized on a high affinity polystyrene microtiter plate. After incubation, plates were washed, and bound antibody was detected by goat anti-human IgG conjugated to horseradish peroxidase (HRP) and then visualized with tetramethylbenzidine (TMB). Patient samples having an optical density (OD) value greater than the cutpoint OD were defined as positive. The cutpoint OD is defined as the mean OD of the normal ten individual human serum samples at 1:200 dilution plus 1.96 times the standard deviation of the normal ten of the assay. The mean cutpoint OD of the 9 validation runs was 0.577 with a % CV of 25.5%. The results of this analysis are set forth in Table 2.

Table 2. IL13-PE38QQR Concentration and IgG Antibodies in Serum of Glioma Patients Given IL13-PE38QQR by CED

Patient Number	Dose	Pre-Infusion		Post-Infusion	
		Titer	Result	Titer	Result
1101	0.5 µg/mL x 4 day	< 200	Negative	5400	Positive
1102	0.5 µg/mL x 4 day	< 200	Negative	< 200	Negative
1103	0.5 µg/mL x 5 day	< 200	Negative	200	Positive
1201	0.5 µg/mL x 5 day	< 200	Negative	600	Positive
1202	0.5 µg/mL x 5 day	< 200	Negative	5400	Positive
1203	0.5 µg/mL x 5 day	< 200	Negative	5400	Positive
1204	0.5 µg/mL x 5 day	< 200	Negative	< 200	Negative
1301	0.5 µg/mL x 6 day	ND	ND	16200	Positive
1302	0.5 µg/mL x 6 day	< 200	Negative	1800	Positive
1303	0.5 µg/mL x 6 day	< 200	Negative	5400	Positive
1401	0.5 µg/mL x 7 day	< 200	Negative	< 200	Negative
1402	0.5 µg/mL x 7 day	200	Positive	200	Positive
1403	0.5 µg/mL x 7 day	< 200	Negative	16200	Positive
1501	1.0 µg/mL x 7 day	200	Positive	16200	Positive*

1502	1.0 µg/mL x 7 day	< 200	Negative	< 200	Negative
1503	1.0 µg/mL x 7 day	200	Positive	5400	Positive*
1601	2.0 µg/mL x 7 day	< 200	Negative	16200	Positive
1602	2.0 µg/mL x 7 day	< 200	Negative	1800	Positive
1603	2.0 µg/mL x 7 day	200	Positive	ND	ND
1701	3.0 µg/mL x 7 day	< 200	Negative	16200	Positive
1702	3.0 µg/mL x 7 day	< 200	Negative	16200	Positive
1703	3.0 µg/mL x 7 day	200	Positive	48600	Positive*
1801	0.5 µg/mL x 4 day	< 200	Negative	5400	Positive
1802	0.5 µg/mL x 4 day	< 200	Negative	< 200	Negative
1803	0.5 µg/mL x 4 day	200	Positive	145800	Positive*

ND = No Data

* = Titer increased 2 orders of magnitude after IL13PE38QQR treatment

[0041] IL13-PE38QQR was not detected in serum of any patient immediately after convection-enhanced delivery of the conjugate. Twenty-five percent of the evaluable patients had low levels of IgG antibodies to IL13-PE38QQR prior to drug administration as determined by screening qualitative ELISA. Seventy-nine percent of the evaluable patients in the study developed IgG antibodies to IL-13PE38QQR after conjugate administration.

[0042] All references, including publications, patent applications, and patents, cited herein are hereby incorporated by reference to the same extent as if each

reference were individually and specifically indicated to be incorporated by reference and were set forth in its entirety herein.

[0043] The use of the terms "a" and "an" and "the" and similar referents in the context of describing the invention (especially in the context of the following claims) are to be construed to cover both the singular and the plural, unless otherwise indicated herein or clearly contradicted by context. The terms "comprising," "having," "including," and "containing" are to be construed as open-ended terms (i.e., meaning "including, but not limited to,") unless otherwise noted. Recitation of ranges of values herein are merely intended to serve as a shorthand method of referring individually to each separate value falling within the range, unless otherwise indicated herein, and each separate value is incorporated into the specification as if it were individually recited herein. All methods described herein can be performed in any suitable order unless otherwise indicated herein or otherwise clearly contradicted by context. The use of any and all examples, or exemplary language (e.g., "such as") provided herein, is intended merely to better illuminate the invention and does not pose a limitation on the scope of the invention unless otherwise claimed. No language in the specification should be construed as indicating any non-claimed element as essential to the practice of the invention.

[0044] Preferred embodiments of this invention are described herein, including the best mode known to the inventors for carrying out the invention. Variations of those preferred embodiments may become apparent to those of ordinary skill in the art upon reading the foregoing description. The inventors expect skilled artisans to employ such variations as appropriate, and the inventors intend for the invention to be practiced otherwise than as specifically described herein. Accordingly, this invention includes all modifications and equivalents of the subject matter recited in the claims appended hereto as permitted by applicable law. Moreover, any combination of the above-described elements in all possible variations thereof is encompassed by the invention unless otherwise indicated herein or otherwise clearly contradicted by context.

WHAT IS CLAIMED IS:

1. A method for inducing an immune response against a disease in a human, the method comprising:
   (a) providing a conjugate comprising a portion of interleukin 13 (IL-13) fused to an immunogen, and
   (b) administering the conjugate to the human, whereupon an immune response against the disease is induced.

2. The method of claim 1, wherein the disease is characterized by expression of IL-13 or expression of an IL-13 receptor.

3. The method of any of claims 1 or 2, wherein the disease is asthma.

4. The method of any of claims 1 or 2, wherein the disease is a cancer selected from the group consisting of glioblastoma, anaplastic astrocytoma (AA), Kaposi sarcoma (KS), renal cell carcinoma (RCC), prostate cancer, head and neck cancer (SCCHN), and ovarian cancer.

5. The method of claim 4, wherein the glioblastoma is malignant glioblastoma multiforme (GBM).

6. The method of any of claims 1-5, wherein the immune response comprises production of one or more antibodies directed against IL-13.

7. The method of claim 6, wherein the one or more antibodies bind to IL-13 in the human and inhibit binding of IL-13 to a receptor for IL-13.

8. The method of claim 1, wherein the portion of IL-13 includes IL-13 and derivatives thereof.

9. The method of any of claims 1-8, wherein the immunogen is selected from a group consisting of bacterial toxin, viral toxin, toxin produced

by a parasitic agent, toxin produced by a plant, cytotoxic drugs, radionuclides and derivatives thereof.

10. The method of claim 9, wherein the bacterial toxin is Pseudomonas aeruginosa exotoxin.

11. The method of any of claims 9-10, wherein the cytotoxicity of the immunogen is reduced or eliminated.

12. The method of any of claims 1-11, wherein the conjugate is IL13-PE38QQR.

13. The method of claim 1, wherein the disease is caused by an infectious agent.

14. The method of claim 13, wherein the infectious agent is selected from the group consisting of a bacterium, a virus, a fungus, or a parasite.

15. The method of any of claims 13-14, wherein the conjugate comprises an immunogen produced by the infectious agent.

16. The method of claim 1, wherein the disease is caused by a cancerous cell.

17. The method of claim 16, wherein the conjugate comprises an immunogen produced by the cancerous cell.

18. The method of any of claims 13-17, wherein the cytotoxicity of the immunogen is reduced or eliminated.

19. The method of claim 18, wherein the immunogen is selected from a group consisting of bacterial toxin, viral toxin, toxin produced by a

parasitic agent, toxin produced by a plant, cytotoxic drugs, radionuclides and derivatives thereof.

20. The method of claim 19, wherein the bacterial toxin is Pseudomonas aeruginosa exotoxin.

21. The method of any of claims 13-20, wherein the immune response comprises production of one or more antibodies directed against the immunogen.

22. The method of any of claims 1-21, wherein the antibody comprises immunoglobins, immunoglobin fragments, multimeric antibodies, immunoglobulin chimeras or mixtures thereof.

23. The method of any of claims 13-22, wherein the conjugate is designated IL13-PE38QQR.

24. The method of any of claims 1-23, wherein the conjugate is a vaccine.

25. The method of any of claims 1-24, wherein the conjugate is administered with a physiologically acceptable carrier.

26. The method of any of claims 1-25, wherein the conjugate is in a neutral or salt form.

27. The method of any of claims 1-26, wherein the conjugate is administered via inhalation.

28. The method of any of claims 1-26, wherein the conjugate is administered parenterally.

29. The method of any of claims 1-26, wherein the conjugate is administered intratumorally.

30. The method of any of claims 1-26, wherein the conjugate is administered intravenously.

31. The method of any of claims 1-26, wherein the conjugate is administered intraperitoneally.

32. The method of any of claims 1-26, wherein the conjugate is administered subcutaneously.

33. A method for treating a disease in a human, the method comprising:
(a) preparing an antibody raised against a conjugate comprising a portion of IL-13 fused to an immunogen, and
(b) administering the antibody to the human, whereupon the disease is treated.

34. The method of claim 33, wherein the disease is asthma.

35. The method of claim 33, wherein the disease is a cancer selected from the group consisting of glioblastoma, anaplastic astrocytoma (AA), Kaposi sarcoma (KS), renal cell carcinoma (RCC), prostate cancer, head and neck cancer (SCCHN), and ovarian cancer.

36. The method of claim 35, wherein the glioblastoma is malignant glioblastoma multiforme (GBM).

37. The method of any of claims 33, wherein the disease is characterized by expression of IL-13 or expression of an IL-13 receptor.

38. The method of claim 33, wherein the portion of IL-13 includes IL-13 and derivatives thereof.

39. The method of claim 33, wherein the antibody is directed against the IL-13 portion of the conjugate.

40. The method of claim 33, wherein the one or more antibodies bind to IL-13 in the human and inhibit binding of IL-13 to a receptor for IL-13.

41. The method of any of claims 39-40, wherein the antibody comprises immunoglobins, immunoglobin fragments, multimeric antibodies, immunoglobulin chimeras or mixtures thereof.

42. The method of claim 33, wherein the disease is caused by an infectious agent.

43. The method of claim 42, wherein the infectious agent is selected from the group consisting of a bacterium, a virus, a fungus, or a parasite.

44. The method of any of claims 42-43, wherein the conjugate comprises an immunogen produced by the infectious agent.

45. The method of claim 33, wherein the disease is caused by a cancerous cell.

46. The method of claim 45, wherein the conjugate comprises an immunogen produced by the cancerous cell.

47. The method of any of claims 42-46, wherein the immunogen is selected from a group consisting of bacterial toxin, viral toxin, toxin

produced by a parasitic agent, toxin produced by a plant, cytotoxic drugs, radionuclides and derivatives thereof.

48. The method of claim 47, wherein the bacterial toxin is Pseudomonas aeruginosa exotoxin.

49. The method of any of claims 33-48, wherein the cytotoxicity of the immunogen is reduced or eliminated.

50. The method of any of claims 42-49, wherein the antibody is directed against the immunogen portion of the conjugate.

51. The method of claim 50, wherein the antibody comprises immunoglobins, immunoglobin fragments, multimeric antibodies, immunoglobulin chimeras or mixtures thereof.

52. The method of any of claims 1-51, wherein the antibody is directed against the immunogen portion and the IL-13 portion of the conjugate.

53. The method of any of claims 33-52, wherein the antibody is administered with a physiologically acceptable carrier.

54. The method of any of claims 33-53, wherein the antibody is in a neutral or salt form.

55. The method of any of claims 33-54, wherein the antibody is administered via inhalation.

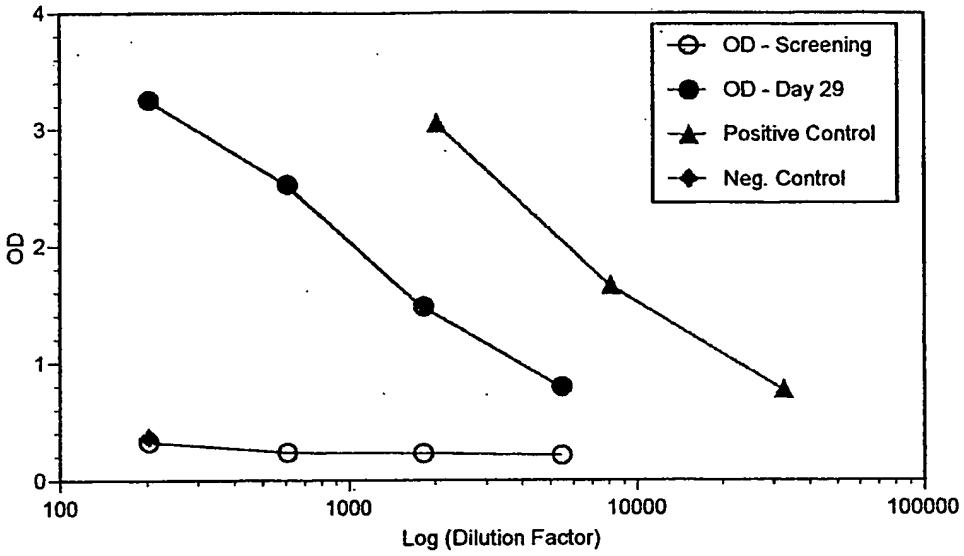
56. The method of any of claims 33-54, wherein the antibody is administered parenterally.

57. The method of any of claims 33-54, wherein the conjugate is administered intratumorally.

58. The method of any of claims 33-54, wherein the conjugate is administered intravenously.

59. The method of any of claims 33-54, wherein the conjugate is administered intraperitoneally.

60. The method of any of claims 33-54, wherein the conjugate is administered subcutaneously.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